TITLE: **METHODS OF INHIBITING DESICCATION OF CUTTINGS REMOVED FROM ORNAMENTAL PLANTS**

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METHODS OF INHIBITING DESICCATION OF CUTTINGS REMOVED FROM ORNAMENTAL PLANTS

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/248,169, filed November 13, 2000, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention generally relates to methods of treating ornamental plants or cuttings removed therefrom to inhibit desiccation of cuttings removed from the ornamental plants.

BACKGROUND OF THE INVENTION

According to an April 2001 report by the United States Department of Agriculture, National Agricultural Statistics Service, Sp Cr 6-1 (01), entitled "Floriculture Crops: 2000 Summary", during the previous year the wholesale value of domestically produced cut flowers was $427 million. The top three valued cut flower categories were Roses at $69.4 million, Lilies at $58.6 million, and Gladioli at $32.2 million. While the U.S. cut flower industry is not insignificant, two-thirds of the cut flowers sold in the U.S. in 1998 were imported, and this import market was worth $1 billion. Of the imports coming into the U.S. that year, 56% were from Colombia, 22% from elsewhere in Central & South America, and about 18% from The Netherlands.

Postharvest handling methods that were developed over 20 years ago on U.S. produced flowers are still current practice in the fresh flower industry. However, as noted above, many flowers sold in the U.S. today are imported from Colombia and Ecuador and can be 8-10 days old when purchased by consumers. Current problems with cut flower longevity and quality are associated with shifts in the geographical locations of production, introduction of new varieties, long-distance transport from farm to consumer, improper transport and storage temperatures, and undesirable handling practices. With respect to transport and storage temperatures,

prevalent problems include: flowers are often not pre-cooled adequately when they leave the grower; use of non-refrigerated trucks during shipment; boxed flowers which sit for extended periods on non-refrigerated docks; and flowers are not kept cool during air transport.

The effect that these problems can have on cut flower longevity includes not only poor appearance of flowers at retail sites, but also loss of flowers (i.e., wilting or dying) prior to the time they reach the retailer or shortly thereafter. In either case, the wholesaler or the retailer may realize financial losses as a result.

A number of strategies have been devised to minimize flower loss. These include treatment with silver thiosulfate, 1-methylcyclopropene (MCP), carboxymethoxylamine (also known as aminooxyacetic acid (AOAA)), AVG, N-AVG, rhizobitoxine, or L-trans-2-amino-4-methoxy-3-butenoic acid (MVG). Silver thiosulfate and MCP are believed to inhibit the effect of either internal or external ethylene, while the others are believed to act internally to inhibit the ability of the cut flowers, plants, and fruit to produce ethylene. These compounds (except MCP) are typically applied to plants or plant materials in the form of an aqueous treatment solution. Applications of the treatment solution to potted plants are carried out by spraying it onto the aerial parts of the plants or by including it in the irrigation water which is supplied to their roots. Treatment of cut flowers or greens is typically carried out by immersing the cut ends of the stems in the aqueous solution containing the treating agent immediately after harvest, during transportation or while the floral arrangement is on display, although they might be treated by immersing the whole flowers into a solution or by spraying them. Since MCP is a gas, it cannot readily be applied in aqueous solution, so plants are treated by exposing them to a modified, controlled atmosphere (containing a defined amount of MCP) in an enclosed chamber.

Silver thiosulfate is expensive and it may be toxic to animals. Although MCP is now commercially available, its use is limited due to difficulties in application and its lack of stability.

However effective these earlier attempts to reduce cut flower losses, there still exists a need to provide improved, non-toxic and easily practiced approaches for minimizing the losses of ornamental plant cuttings. The present invention is directed to overcoming these deficiencies in the art.

SUMMARY OF THE INVENTION

A first aspect of the present invention relates to a method of inhibiting desiccation of cuttings from ornamental plants which includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit desiccation of a cutting from the ornamental plant after the cutting is removed from the ornamental plant.

A second aspect of the present invention relates to a cutting which has been removed from an ornamental plant treated with a hypersensitive response elicitor protein or polypeptide, wherein the cutting is characterized by greater resistance to desiccation as compared to a cutting removed from an untreated ornamental plant.

A third aspect of the present invention relates to a method of promoting early flowering of an ornamental plant which includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide under conditions effective to promote early flowering of the ornamental plant.

A fourth aspect of the present invention relates to a method of harvesting a cutting from an ornamental plant which includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide and harvesting a cutting from the treated ornamental plant.

A fifth aspect of the present invention relates to a method of harvesting a cutting from an ornamental plant which includes: harvesting a cutting from an ornamental plant and treating the harvested cutting with a hypersensitive response elicitor protein or polypeptide.

A sixth aspect of the present invention relates to a method of inhibiting desiccation of cuttings from ornamental plants which includes: removing a cutting from an ornamental plant and treating the removed cutting with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit desiccation of the removed cutting.

A seventh aspect of the present invention relates to a cutting which has been removed from an ornamental plant, wherein the cutting has been treated with a hypersensitive response elicitor protein or polypeptide and wherein the cutting is characterized by greater resistance to desiccation as compared to an untreated cutting removed from the ornamental plant.

An eight aspect of the present invention relates to a method of inhibiting desiccation of cuttings from ornamental plants which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic ornamental plant or transgenic ornamental plant produced from the transgenic ornamental plant seed under conditions effective to inhibit desiccation in a cutting removed from the transgenic plant.

A ninth aspect of the present invention relates to a method of promoting early flowering of an ornamental plant which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic ornamental plant or transgenic ornamental plant produced from the transgenic ornamental plant seed under conditions effective to promote early flowering of the transgenic ornamental plant.

A tenth aspect of the present invention relates to a method of harvesting a cutting from an ornamental plant which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein; growing the transgenic ornamental plant or transgenic ornamental plant produced from the transgenic ornamental plant seed under conditions; and harvesting a cutting from the grown transgenic ornamental plant, wherein the cutting exhibits a reduced susceptibility to desiccation as compared to cuttings removed from non-transgenic ornamental plants.

An eleventh aspect of the present invention relates to a cutting which has been removed from a transgenic ornamental plant which expresses a heterologous hypersensitive response elicitor protein or polypeptide, wherein the cutting is characterized by greater resistance to desiccation as compared to a cutting removed from a non-transgenic ornamental plant.

A twelfth aspect of the present invention relates to a method of enhancing the longevity of flower blooms on ornamental plant cuttings which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic ornamental plant or transgenic ornamental plant produced

from the transgenic ornamental plant seed under conditions effective to enhancing the longevity of flower blooms on cuttings removed therefrom.

A thirteenth aspect of the present invention relates to a method of enhancing the longevity of flower blooms on ornamental plant cuttings which includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhancing the longevity of flower blooms on cuttings removed therefrom.

A fourteenth aspect of the present invention relates to a method of enhancing the longevity of flower blooms on ornamental plant cuttings which includes: harvesting a cutting from an ornamental plant and treating the harvested cutting with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhancing the longevity of flower blooms on the harvested cutting.

Because hypersensitive response elicitor proteins or polypeptides can easily be expressed transgenically in or applied topically to ornamental plants and/or ornamental plant cuttings, the present invention offers an effective, simple-to-use, non-toxic approach for inhibiting the desiccation of cuttings removed from ornamental plants, promoting early flowering of the ornamental plants, and enhancing the longevity of flower blooms on ornamental plant cuttings. By inhibiting desiccation of cuttings after they have been removed from an ornamental plant, the cuttings are less likely to wilt and die before they are received by the retailer. This will dramatically decrease losses associated with long transportation rates in less than ideal conditions. Moreover, it is also possible to enhancing the longevity of flower blooms, which end consumers can clearly appreciate.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an image illustrating the response of *Vega* roses to pre- and postharvest application of EBC-151 (left), untreated (center), and preharvest only treatment with EBC-151. Image captured 16 days after harvest and postharvest treatment with EBC-151.

Figure 2 is an image illustrating the response of *Vega* roses to pre-harvest only applications of EBC-151; 150 + 350 g/Ha (left), untreated (center), and

250 g/Ha (right). Image captured 16 days after harvest; no postharvest treatment applied.

Figure 3 is an image illustrating the response of *Vega* roses to postharvest only application of EBC-151. Image captured 16 days after harvest.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of inhibiting desiccation of cuttings from ornamental plants, methods of harvesting cuttings from ornamental plants, methods of promoting early flowering of ornamental plants, and methods of enhancing the longevity of flower blooms on ornamental plant cuttings.

The ornamental plants can be transgenic plants which express a heterologous hypersensitive response elicitor protein or polypeptide or the ornamental plants can be treated (i.e., via topical application) with a hypersensitive response elicitor protein or polypeptide. Alternatively, the cutting from the ornamental plant (whether transgenic or not) can itself be treated with a hypersensitive response elicitor protein or polypeptide, independent of any treatment provided to the ornamental plant from which the cutting is removed.

For use in accordance with these methods, suitable hypersensitive response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from *Erwinia* species (e.g., *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*), *Ralstonia* species (e.g., *Ralstonia solanacearum*), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response elicitor derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitins) from various *Phytophthora* species (e.g., *Phytophthora parasitica,*

Phytophthora cryptogea, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

Preferably, the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi, Erwinia amylovora, Pseudomonas syringae, Ralstonia solanacearum*, or *Xanthomonas campestris*.

A hypersensitive response elicitor protein or polypeptide from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                   10                  15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20                  25                  30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35                  40                  45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50                  55                  60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
                85                  90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100                 105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
        115                 120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
    130                 135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145                 150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180                 185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
        195                 200                 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
    210                 215                 220
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Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225                 230                 235                 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
                245                 250                 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
            260                 265                 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
        275                 280                 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
    290                 295                 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305                 310                 315                 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
                325                 330                 335

Asn Ala
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This hypersensitive response elicitor protein or polypeptide has a molecular mass of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

```
cgattttacc cgggtgaacg tgctatgacc gacagcatca cggtattcga caccgttacg   60
gcgtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgtc  120
gatctggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccgggg  180
cagcaatatc ccggcatgtt gcgcacgctg ctcgctcgtc gttatcagca ggcggcagag  240
tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg  300
ccgtcggatc ccggcagtta tccgcaggtg atcgaacgtt tgtttgaact ggcgggaatg  360
acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc  420
cgatcattaa gataaaggcg gctttttta ttgcaaaacg gtaacggtga ggaaccgttt  480
caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgtg  540
ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca  600
aattacgatc aaagcgcaca tcggcggtga tttgggcgtc tccggtctgg ggctgggtgc  660
tcagggactg aaaggactga attccgcggc ttcatcgctg ggttccagcg tggataaact  720
gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct  780
ggcgcagggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc  840
tttcggcaat ggcgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcggcga  900
tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac  960
caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020
ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080
caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggctt 1140
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gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200
ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260
ccgccacttt gtagataaag aagatcgcgg catggcgaaa gagatcggcc agtttatgga 1320
tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380
gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440
cgccagcatg gacaaattcc gtcaggcgat gggtatgatc aaaagcgcgg tggcgggtga 1500
taccggcaat accaacctga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560
ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620
atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680
ttattatgcg gtttatgcgg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740
acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800
gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860
cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccctttag 1920
cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980
gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040
aaaatagggc agtttttgcg tggtatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100
gttcgtcatc atctttctcc atctgggcga cctgatcggt t                     2141
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The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,850,015 to Bauer et al. and U.S. Patent No. 5,776,889 to Wei et al., each of which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

```
Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1               5                   10                  15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
            20                  25                  30

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
        35                  40                  45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
    50                  55                  60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65                  70                  75                  80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
                85                  90                  95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
            100                 105                 110
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Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
165 170 175

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
180 185 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
195 200 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
210 215 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225 230 235 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
245 250 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
260 265 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
275 280 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
290 295 300

Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305 310 315 320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
325 330 335

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
355 360 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385 390 395 400

Gly Ala Ala

This hypersensitive response elicitor protein or polypeptide has a molecular mass of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```
aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa     60
gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct    120
atcggcggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg    180
ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg    240
gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcggtgg tgggctgatg    300
ggcggtggct taggcggtgg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa    360
ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa    420
ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggtattaac    480
tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac    540
ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggtgatggg    600
caagatggca cccagggcag ttcctctggg ggcaagcagc cgaccgaagg cgagcagaac    660
gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag    720
ctccttggca acgggggact gggaggtggt cagggcggta atgctggcac gggtcttgac    780
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag    840
ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat    900
atcggtacgc acaggcacag ttcaacccgt tctttcgtca ataaaggcga tcgggcgatg    960
gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac   1020
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc   1080
aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc   1140
atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc   1200
ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca   1260
cttggcaagc tgggcgcggc ttaagctt                                      1288
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The above nucleotide and amino acid sequences are disclosed are further described in U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al., each of which is hereby incorporated by reference in its entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

```
Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1               5                   10                  15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
            20                  25                  30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
        35                  40                  45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
    50                  55                  60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65                  70                  75                      80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
                85                  90                  95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
            100                 105                 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
        115                 120                 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
    130                 135                 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145                 150                 155                     160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
                165                 170                 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
            180                 185                 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
        195                 200                 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
    210                 215                 220

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225                 230                 235                     240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
                245                 250                 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
            260                 265                 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
        275                 280                 285
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Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
    290                 295                 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305                 310                 315                 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
                325                 330                 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
            340                 345                 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
        355                 360                 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
    370                 375                 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385                 390                 395                 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
                405                 410                 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
            420                 425                 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
        435                 440                 445
```

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular mass of ca. 45 kDa. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

```
atgtcaattc ttacgcttaa caacaatacc tcgtcctcgc cgggtctgtt ccagtccggg    60
ggggacaacg ggcttggtgg tcataatgca aattctgcgt tggggcaaca acccatcgat   120
cggcaaacca ttgagcaaat ggctcaatta ttggcggaac tgttaaagtc actgctatcg   180
ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggt   240
aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa ccactccgca gtctgacagt   300
cagaacatgc tgagtgagat gggcaacaac gggctggatc aggccatcac gcccgatggc   360
cagggcggcg ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca   420
cgcatgatgg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc   480
tcttccggta cttcttcatc tggcggttcc ccttttaacg atctatcagg ggggaaggcc   540
ccttccggca actccccttc cggcaactac tctcccgtca gtaccttctc acccccatcc   600
acgccaacgt cccctacctc accgcttgat ttcccttctt ctcccaccaa agcagccggg   660
ggcagcacgc cggtaaccga tcatcctgac cctgttggta gcgcgggcat cggggccgga   720
```

```
aattcggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc  780
gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta  840
ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc  900
ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcatcttta cggtgatgcc  960
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020
agcgcgggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080
aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac 1140
tttggtactt ttgtacgcac taacggcggt caacagggta actgggatct gaatctgagc 1200
catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac 1260
gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320
gccaacctga aggtggctga atga                                        1344
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 6,262,018 to Kim et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

```
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1               5                   10                  15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
            20                  25                  30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
        35                  40                  45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
    50                  55                  60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65                  70                  75                  80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
                85                  90                  95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
            100                 105                 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
        115                 120                 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
    130                 135                 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145                 150                 155                 160
```

```
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
                165                 170                 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
            180                 185                 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195                 200                 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210                 215                 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225                 230                 235                 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
                245                 250                 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
            260                 265                 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275                 280                 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290                 295                 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305                 310                 315                 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
                325                 330                 335

Asn Gln Ala Ala Ala
            340
```

This hypersensitive response elicitor protein or polypeptide has a molecular mass of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

```
atgcagagtc tcagtcttaa cagcagctcg ctgcaaaccc cggcaatggc ccttgtcctg    60
gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc   120
gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga   180
```

```
aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc    240
atcgctgcgc tggacaagct gatccatgaa aagctcggtg acaacttcgg cgcgtctgcg    300
gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc    360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac    420
gatatgccga tgctgaacaa gatcgcgcag ttcatggatg acaatcccgc acagtttccc    480
aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac    540
gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag    600
agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc    660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc    720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa    780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtacgtcg    840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag    900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct    960
gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca   1020
gcctga                                                              1026
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et al., each of which is hereby incorporated by reference in its entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```
Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1               5                   10                  15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
            20                  25                  30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
        35                  40                  45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
    50                  55                  60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65                  70                  75                  80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
                85                  90                  95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
            100                 105                 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
        115                 120                 125
```

```
Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
    130                 135                 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
                165                 170                 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
            180                 185                 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
        195                 200                 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
    210                 215                 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225                 230                 235                 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
                245                 250                 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
            260                 265                 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
        275                 280                 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
    290                 295                 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305                 310                 315                 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
                325                 330                 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
            340                 345                 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
        355                 360                 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
    370                 375                 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385                 390                 395                 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
                405                 410                 415

Ala Ser Thr Gln His Thr Glu Leu
            420
```

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive

response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

```
tccacttcgc tgattttgaa attggcagat tcatagaaac gttcaggtgt ggaaatcagg   60
ctgagtgcgc agatttcgtt gataagggtg tggtactggt cattgttggt catttcaagg  120
cctctgagtg cggtgcggag caataccagt cttcctgctg gcgtgtgcac actgagtcgc  180
aggcataggc atttcagttc cttgcgttgg ttgggcatat aaaaaaagga acttttaaaa  240
acagtgcaat gagatgccgg caaaacggga accggtcgct gcgctttgcc actcacttcg  300
agcaagctca accccaaaca tccacatccc tatcgaacgg acagcgatac ggccacttgc  360
tctggtaaac cctggagctg gcgtcggtcc aattgcccac ttagcgaggt aacgcagcat  420
gagcatcggc atcacacccc ggccgcaaca gaccaccacg ccactcgatt tttcggcgct  480
aagcggcaag agtcctcaac caaacacgtt cggcgagcag aacactcagc aagcgatcga  540
cccgagtgca ctgttgttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga  600
cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc  660
taaattgatc agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa  720
gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct  780
cggtacaccg tcggccgata gcgggggcgg cggtacaccg gatgcgacag gtggcggcgg  840
cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg  900
tggcggcagc ggtggcggcg gcacacccac tgcaacaggt ggcggcagcg gtggcacacc  960
cactgcaaca ggcggtggcg agggtggcgt aacaccgcaa atcactccgc agttggccaa 1020
ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagatc aatgtggtga aagacaccat caaggtcggc gctggcgaag tctttgacgg 1140
ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260
cgaggtcgat ggcatccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320
gcatgcccag aacgtcggtg aagacctgat tacggtcaaa ggcgagggag gcgcagcggt 1380
cactaatctg aacatcaaga acagcagtgc caaaggtgca gacgacaagg ttgtccagct 1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt 1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc 1560
taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg 1620
caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgacccaaca 1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc             1729
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 6,172,184 to Collmer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Ralstonia solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5                   10                  15
```

```
Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20                  25                  30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35                  40                  45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
    50                  55                  60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
                85                  90                  95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100                 105                 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
        115                 120                 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
    130                 135                 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145                 150                 155                 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335
```

```
Gln Ser Thr Ser Thr Gln Pro Met
                340
```

Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Ralstonia solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its entirety. It is encoded by a DNA molecule from *Ralstonia solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

```
atgtcagtcg gaaacatcca gagcccgtcg aacctcccgg gtctgcagaa cctgaacctc    60
aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc   120
gagaaggaca tcctcaacat catcgcagcc ctcgtgcaga aggccgcaca gtcggcgggc   180
ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgccaa cgcgggcgcc   240
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc   300
ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa   360
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag   420
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc   480
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcggcgg tgctggcgcc   540
ggcggcgcgg gtggcggtgt cggcggtgct ggtggcgcgg atggcggctc cggtgcgggt   600
ggcgcaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac   660
ggcccgcaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac   720
cagggcggcc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag   780
atgatgcagc aaggcggcct cggcggcggc aaccaggcgc agggcggctc gaagggtgcc   840
ggcaacgcct cgccggcttc cggcgcgaac ccgggcgcga accagcccgg ttcggcggat   900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc   960
gtccagatcc tgcagcagat gctggcggcg cagaacggcg gcagccagca gtccacctcg  1020
acgcagccga tgtaa                                                   1035
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Xanthomonas campestris* has an amino acid sequence corresponding to SEQ. ID. No. 13 as follows:

```
Met Asp Ser Ile Gly Asn Asn Phe Ser Asn Ile Gly Asn Leu Gln Thr
 1               5                  10                  15
```

```
Met Gly Ile Gly Pro Gln Gln His Glu Asp Ser Ser Gln Gln Ser Pro
            20                  25                  30

Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile
        35                  40                  45

Met Met Met Leu Gln Gln Ser Gln Gly Ser Asp Ala Asn Gln Glu Cys
    50                  55                  60

Gly Asn Glu Gln Pro Gln Asn Gly Gln Gln Glu Gly Leu Ser Pro Leu
65                  70                  75                  80

Thr Gln Met Leu Met Gln Ile Val Met Gln Leu Met Gln Asn Gln Gly
                85                  90                  95

Gly Ala Gly Met Gly Gly Gly Gly Ser Val Asn Ser Ser Leu Gly Gly
            100                 105                 110

Asn Ala
```

This hypersensitive response elicitor protein has an estimated molecular mass of about 12 kDa based on the deduced amino acid sequence, which is consistent with the molecular mass of about 14 kDa as detected by SDS-PAGE. It is encoded by a DNA molecule from *Xanthomonas campestris* having a nucleotide sequence corresponding SEQ. ID. No. 14 as follows:

```
atggactcta tcggaaacaa cttttcgaat atcggcaacc tgcagacgat gggcatcggg  60
cctcagcaac acgaggactc cagccagcag tcgccttcgg ctggctccga gcagcagctg 120
gatcagttgc tcgccatgtt catcatgatg atgctgcaac agagccaggg cagcgatgca 180
aatcaggagt gtggcaacga acaaccgcag aacggtcaac aggaaggcct gagtccgttg 240
acgcagatgc tgatgcagat cgtgatgcag ctgatgcaga accagggcgg cgccggcatg 300
ggcggtggcg gttcggtcaa cagcagcctg ggcggcaacg cc                    342
```

The above protein and nucleic acid molecule are further described in U.S. Patent Application Serial No. 09/412,452 to Wei et al., filed April 9, 2001, which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of hypersensitive response elicitor proteins or polypeptides derived from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of an *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves,"

MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety. A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, each of which is hereby incorporated by reference in its entirety.

Hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), each of which is hereby incorporated by reference in its entirety.

Another hypersensitive response elicitor protein or polypeptide which can be used in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus* and is described in U.S. Patent Application Serial No. 09/136,625 to Beer et al., filed August 19, 1998, which is hereby incorporated by reference in its entirety.

Fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens can also be used according to the present invention.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory,

Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), each of which is hereby incorporated by reference in its entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ.

ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in a chimeric gene of the present invention.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor proteins or polypeptides used in accordance with the present invention are preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the hypersensitive response elicitor protein or polypeptide of interest is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC. Alternatively, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells (discussed *infra*) and removed therefrom.

One particular hypersensitive response elicitor protein, known as harpin$_{Ea}$, is commercially available from Eden Bioscience Corporation (Bothell, Washington) under the name of Messenger®. Messenger® contains 3% by weight of harpin$_{Ea}$ as the active ingredient and 97% by weight inert ingredients. Harpin$_{Ea}$ is one

type of hypersensitive response elicitor protein from *Erwinia amylovora*, identified herein by SEQ. ID. No. 3.

Other hypersensitive response elicitors can be readily identified by isolating putative protein or polypeptide candidates and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art.

DNA molecules encoding other hypersensitive response elicitor proteins or polypeptides can also be identified by determining whether such DNA molecules hybridizes under stringent conditions to a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, or 14. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 μg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an

expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems

infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *rec*A promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*,

and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

Because it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide, it is preferable that the host cell also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System

Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety.

Isolation of the hypersensitive response elicitor protein or polypeptide from the host cell or growth medium can be carried out as described above.

The methods of the present invention can be performed by treating the ornamental plant or a cutting removed therefrom.

Before removal of a cutting, suitable application methods include, without limitation, high or low pressure spraying of the entire plant. After removal of a cutting, suitable application methods include, without limitation, low or high pressure spraying, coating, or immersion. Other suitable application procedures (both pre- and post-cutting) can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor protein or polypeptide with the cutting. Once treated, the cuttings can be handled, packed, shipped, and processed using conventional procedures to deliver the cuttings to distributors or end-consumers.

The hypersensitive response elicitor polypeptide or protein can be applied to cuttings in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to cuttings with other materials being applied at different times.

A composition suitable for treating ornamental plants or cuttings therefrom in accordance with the application embodiment of the present invention contains an isolated hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. The composition preferably contains greater than about 500 nM hypersensitive response elicitor polypeptide or protein, although greater or lesser amounts of the hypersensitive response elicitor polypeptide or protein depending on the rate of composition application and efficacy of different hypersensitive response elicitor proteins or polypeptides.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof.

Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and ripening agents. These materials can be used either to facilitate the process of the present invention or to provide additive benefits to inhibit desiccation or promote flowering.

As indicated above, one embodiment of the present invention involves treating ornamental plants or their cuttings with an isolated hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor protein or polypeptide can be isolated from its natural source (e.g., *Erwinia amylovora, Pseudomonas syringae*, etc.) or from recombinant source transformed with a DNA molecule encoding the protein or polypeptide.

Another aspect of the present invention relates to a DNA construct as well as host cells, expression systems, and transgenic plants which contain the heterologous DNA construct.

The DNA construct includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule and which is effective to transcribe the DNA molecule in the tissues of cuttings, and a 3' regulatory region operably coupled to the DNA molecule. Expression of the DNA molecule in such tissues imparts to a cutting resistance against desiccation.

Expression of such heterologous DNA molecules requires a suitable promoter which is operable in plant tissues. In some embodiments of the present invention, it may be desirable for the heterologous DNA molecule to be expressed in many, if not all, tissues. Such promoters yield constitutive expression of coding sequences under their regulatory control. Exemplary constitutive promoters include, without limitation, the nopaline synthase promoter (Fraley et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety). Other constitutive plant promoters are continuously being identified and can be used in accordance with the present invention.

While constitutive expression is generally suitable for expression of the DNA molecule, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with the DNA molecule that are expressed at only certain stages of development or only in certain tissues.

In another embodiment of the present invention, expression of the heterologous DNA molecule is directed in a tissue-specific manner or environmentally-regulated manner (i.e., inducible promoters). Tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

Promoters useful for expression in flower tissues include the 5-enolpyruvylshikimate-3-phosphate synthase promoter (Benfy, et al., "Sequence Requirements of the 5-enolpyruvylshikimate-3-phosphate Synthase 5'-Upstream Region for Tissue-Specific Expression in Flowers and Seedlings," The Plant Cell 2:849-856 (1990), which is hereby incorporated by reference in its entirety) and the tomato PG β-subunit promoter (U.S. Patent No. 6,127,179 to DellaPenna et al., which is hereby incorporated by reference).

Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gst1* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., each of which is hereby incorporated by reference in its entirety.

Expression of the DNA molecule in isolated plant cells or tissue or whole plants also utilizes appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the first and second DNA molecules. A number of 3' regulatory

regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

The promoter and a 3' regulatory region can readily be ligated to the DNA molecule using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

One approach to transforming plant cells with a DNA molecule of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., each of which is hereby incorporated by reference in its entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the DNA molecule into plant cells is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the DNA molecule. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference in its entirety. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the DNA molecule. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the DNA molecule. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a DNA molecule a hypersensitive response elicitor protein or polypeptide can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference in its entirety.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the DNA molecule of

the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic ornamental plant that includes a heterologous DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, wherein the heterologous DNA molecule is under control or a promoter that induces transcription of the DNA molecule in tissues of cuttings. Preferably, the DNA molecule is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics is hereby incorporated by reference in its entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including both monocots and dicots.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If

these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the DNA molecule encoding the hypersensitive response elicitor protein or polypeptide is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

With respect to desiccation, complete protection against desiccation may not be conferred, but the severity of desiccation can be reduced. Desiccation protection inevitably will depend, at least to some extent, on other conditions such as storage temperatures, light exposure, etc. However, this method of controlling desiccation has the potential for eliminating some other treatments (i.e., additives to water, thermal regulation, etc.) which may contribute to reduced costs or, at least, substantially no increase in costs. Moreover, by controlling desiccation, it is also possible to enhance the longevity of flower blooms.

The methods of the present invention can be utilized to treat a wide variety of ornamental plants to control desiccation of cuttings removed therefrom as well as enhance the longevity of flowers. Ornamental plants can be either monocots or dicots. Cuttings include stems, leaves, flowers, or combinations thereof.

In addition to treatment with hypersensitive response elicitor proteins or polypeptides, as well as transgenic expression thereof in tissues of cuttings, cuttings or ornamental plants (transgenic or otherwise) can also be treated with ethylene action inhibitors of the types disclosed in U.S. Patent No. 6,194,350 to Sisler, U.S. Patent No. 6,153,559 to Heiman, and U.S. Patent No. 5,518,988 to Sisler et al., each of which is hereby incorporated by reference in its entirety. Such treatment can occur before harvest, after harvest, or both. One commercially available ethylene-action inhibitor is EthylBloc® (1-methylcyclopropene, available from AgroFresh Inc. and Floralife Inc.).

EXAMPLES

The following examples are intended to illustrate, but by no means are intended to limit, the scope of the present invention as set forth in the appended claims.

Example 1- Increased Flower Quality and Longevity of Roses from Postharvest Application of EBC-151 (Messenger®)

Mature rose plants were treated with Messenger® (coded as EBC-151) by foliar sprays and postharvest treatment to improve flower quality and longevity. The trial was established in a commercial rose greenhouse in Villa Guerrero, Mexico. The rose variety in this trial was *Vega*. Individual plot beds contained approximately 44 mature plants arranged in two rows; each plot was replicated 4 times and measured 80 cm wide by 15.4 m long. EBC-151 treatments were applied with a CO_2-powered backpack sprayer calibrated to deliver 430 1/Ha at 90 psi. Treatment rates and timings in this trial are shown in Table 1 below.

Table 1: Application rates and treatment schedule for EBC-151 to *Vega* roses

Treatment	EBC-151 Application Rate	Treatment Details
1	250 g/Ha	8 applications at approximately 14-d intervals
2	250 g/Ha + 3.33 g/L postharvest spray	8 applications at approximately 14-d intervals followed by a postharvest spray to 10 commercially-harvested flower/stems within 1 hour of cutting
3	150 g Ha + 350 g/Ha	150 g/Ha applied 5 times followed by 350 g/Ha applied 3 times at the same 14-d schedule, no postharvest application
4	150 g/Ha + 350 g/Ha + 3.33 g/L postharvest spray	150 g/Ha applied 5 times followed by 350 g/Ha applied 3 times at the same 14-d schedule followed by a postharvest spray to 10 commercially-harvested flower/stems within 1 hour of cutting
5	3.33 g/L postharvest spray only	Postharvest spray only to 10 commercially-harvested flower/stems within 1 hour of cutting
6	N/a	Untreated with EBC-151

Preharvest applications of each EBC-151 treatment were repeated at approximately 14-d intervals. After the fifth preharvest application, 10 mature flower/stems were randomly selected from each treatment and evaluated. Treatment effects were evaluated on cut flowers by assessing the number of open flowers and the

number of "straight" stems on each flower/stem. An "open" flower was determined to conform to commercial standards for sale by having flower petals extended. Flower petals judged as partially extended were rated as "not open". Straight stems were evaluated as conforming to commercial standard of acceptability for sale. Results for this evaluation are shown in Table 2 below. No postharvest applications of EBC-151 were made to flower/stems harvested after the fifth application of EBC-151.

Table 2: Response of cut *Vega* roses to treatment with EBC-151 (five applications only)

Treatment	Number of Flowers	Number of "Open" Flowers	Percent "open" Flowers	Number of Flowers with "Straight" Stems
1	10	10	100	10
3	10	2	20	6
6	10	1	10	4

Additional preharvest treatments continued with three more applications (for a total of eight applications). Following the eighth application, an additional 10 mature flower/stems were then randomly selected from each treatment and evaluated in the same manner as had been done after the fifth application. Immediately after cutting (within 1 hour) a single postharvest treatment of EBC-151 was applied at the rate of 3.33 g/L (100 ppm a.i.) to the cut flower/stems harvest from Treatments 2, 4 and 5. The postharvest spray was applied by completely misting each flower/stem with the EBC-151 solution. Sixteen days after postharvest treatment, the number of open flowers and number of flowers with "straight" stems were determined for each treatment. Results for this evaluation are shown in Table 3 below.

Table 3: Response of cut *Vega* roses to treatment with EBC-151
(eight preharvest and one postharvest application)

Treatment	Number of Flowers	Number of "Open" Flowers	Percent "open" Flowers	Number of Flowers with "Straight" Stems
1	10	9	90	8
2	10	10	100	8
3	10	9	90	9
4	10	10	100	9
5	10	3	30	1
6	10	2	20	2

Visual observations of cut roses 16 days after postharvest treatment were made for treatments that received postharvest applications of EBC-151. Roses that had been treated with the postharvest application of EBC-151 appeared to have substantially greater longevity than those that had not received the postharvest treatment (Figures 1-3).

Results of this trial demonstrated a treatment effect for application of EBC-151 (Messenger®) to roses. The effect was seen in a substantially greater increase in the number of open flowers at harvest. This effect is of significant commercial benefit to rose growers. In addition, the postharvest application of EBC-151 to cut roses resulted in substantially extending the "shelf life" of the cut roses.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.